KHMedical

IVD | REF RP033

RADIONE Ebola/Marburg Detection Kit

This instruction must be read carefully prior to use. Reliability of assay results cannot be guaranteed if there is any deviation from the instructions

1. Intended use

The RADIONE Ebola/Marburg Detection Kit is a qualitative, automated in vitro diagnostic test intended to aid in the diagnosis of Ebola virus disease and Marburg virus disease through the detection of Ebola virus RNA and Marburg virus RNA in human EDTA plasma specimens.
The assay is intended for testing symptomatic individuals suspected of Ebola virus or Marburg virus infection based on clinical signs, symptoms, exposure history, and/or applicable epidemiological criteria.
The kit allows for the simultaneous detection of Ebola virus and Marburg virus. Using the fully automated and enclosed RADIONE platform, the test integrates nucleic acid extraction, amplification, and result analysis in a single system.
The assay detects Zaire, Sudan, Bundibugyo, Taï Forest, and Reston ebolaviruses within the validated detection scope and reports them collectively as "Ebola Positive" without differentiation among individual Ebola virus species. Marburg virus is reported separately as "Marburg Positive."
The test is intended for use by trained healthcare or laboratory professionals with the RADIONE 4ch Instrument (KM010) and the RADIONE Universal DNA/RNA Extraction Kit (KP001).

2. Principle of RADIONE system

The RADIONE Ebola/Marburg Detection Kit detects Ebola virus RNA targeting the NP gene region and Marburg virus RNA targeting the VP40 gene region.
Nucleic acids are automatically extracted using a magnetic bead-based method, followed by reverse transcription real-time PCR amplification. Target amplification is detected using sequence-specific fluorescent probes.
Human RNase P is used as an endogenous internal control to monitor specimen adequacy and the validity of the extraction and amplification processes.
The RADIONE software, version NATV1.0.17.12, automatically analyzes fluorescence signals and classifies results as positive, negative, or invalid according to the predefined interpretation criteria provided in the Result Interpretation section.

3. Precautions and Warning

1) This assay should be performed by personnel trained in molecular diagnostic procedures and operation of the RADIONE 4ch (KM010).
2) Wear disposable powder-free gloves and appropriate personal protective equipment when handling reagents and specimens
3) When a control result is outside the expected range described in Section 11, "Quality Control," the test result shall be considered invalid. Do not report the patient result and repeat the test using new reagents.
4) Do not use the kit after its expiration date or if the packaging or reagent components are damaged or leaking.
5) Follow standard precautions for infectious-waste management. All patient specimens and used test material should be considered potentially infectious and handled with appropriate precautions.
6) Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where reagents and human specimens are handled.
7) Handle all specimens as potentially infectious and follow applicable laboratory biosafety procedures [3].
8) Specimen processing should be performed in accordance with applicable national biosafety regulations.
9) Dispose of used kit reagents, cartridges, PCR tubes, and human specimens in accordance with applicable local and national regulations.
10) Do not reuse disposable materials after use. Use a new pipette tip for each specimen and pipetting step to prevent cross-contamination.
11) Do not mix or pool reagents from different lots or from different kits of the same lot.
12) The kit components and required accessories are supplied non-sterile unless otherwise indicated on the label. The extraction cartridge, pre-filled PCR tubes, and other disposable consumables are intended for single use only and must not be reused.
13) Before use, inspect the kit box, aluminum pouch, cartridges, tubes, and other consumables for evidence of damage, leakage, opening, or loss of seal integrity. Do not use any component if its packaging is damaged, opened, leaking, or otherwise compromised. Discard the affected component in accordance with applicable biosafety procedures and use a new, intact component.
14) Do not test specimens that do not meet the validated specimen acceptance criteria described in Section 8. Collect a new specimen when the acceptance criteria are not met.
15) Follow the applicable product label, Safety Data Sheet, and institutional safety procedures for any reagent or consumable requiring specific hazardous-material handling.

4. Kit Components

Materials Provided (24tests/Kit)

Component	Unit
Ebola & Marburg pre-filled PCR tube	4 well-strip tube X 24 EA

5. Materials Required but Not Provided

- RADIONE Universal DNA/RNA Extraction Kit (KP001)
- RADIONE 4ch Instrument (KM010) Cartridge Holder (provided with the equipment)
- Disposable powder-free gloves

6. Instruments and materials Available, but not provided

- RADIONE Ebola/Marburg Positive Control Kit (RP033-PC)
- Mini centrifuge (<4000rpm)

7. Storage & Shelf life

- All components should be stored at -25°C to -15°C upon arrival.
- The kit has a shelf life of 24months from manufacture date.
- Freeze-thaw cycles should not exceed 5 times.
- Components can be stored at 2-8 °C for up to 20 days.
- Components can be stored at 20-25 °C for up to 8 days.

8. Specimen collection, transportation and storage

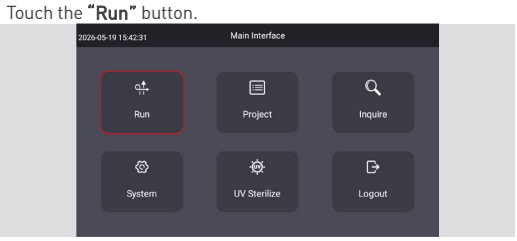
- The validated specimen type for the RADIONE Ebola/Marburg Detection Kit is human EDTA plasma. Specimens other than human EDTA plasma, or specimens that are inadequately identified, insufficient in volume, visibly clotted, leaking, or stored or transported outside the conditions specified in this IFU, must not be used for testing. A new specimen should be collected if the specimen does not meet these acceptance criteria.
- All clinical specimens should be transported in non-breakable, leak-proof transport containers to prevent exposure through leakage. When specimens are transported to another location, applicable national biohazard transport regulations and guidelines must be followed.
- Human EDTA plasma specimens may be stored at 2°C to 8°C for up to 7 days and may undergo up to three freeze-thaw cycles. Each cycle consists of storage at ≤–70°C for at least 12 hours followed by complete thawing at 20°C to 25°C.
- The Specimen collection device manufacturer's protocol and instructions must be followed when collecting whole blood in an EDTA containing tube and preparing plasma specimens.
- Bring refrigerated specimens to room temperature before testing. Completely thaw frozen specimens at 20°C to 25°C, then gently mix by inversion before aliquoting. Verify specimen identification, volume, container integrity, and absence of visible clots. Do not test specimens that do not meet the acceptance criteria in Section 8.
- Collect whole blood using an EDTA anticoagulant collection tube in accordance with the tube manufacturer's instructions. Prepare EDTA plasma by centrifugation according to the laboratory's validated procedure. Do not use serum, whole blood, or plasma collected using anticoagulants other than EDTA.

9. Test Procedure

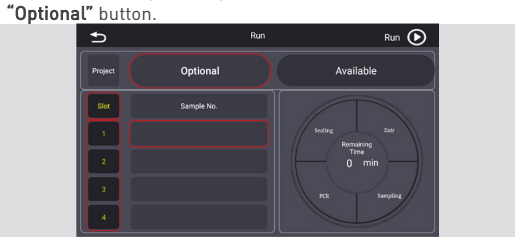
The RADIONE 4ch Instrument contains four independent test slots and can process one to four specimens during a test run. Each specimen is prepared in a separate cartridge and loaded into a corresponding test slot. Only the slot(s) containing a prepared cartridge should be selected in the software. Each selected slot is assigned its own Sample No. and test result.

9-1 Equipment and software setup

1) Turn on the **RADIONE 4ch** instrument.
2) Enter the username and password to log in.
3) Touch the **"Run"** button.



4) Enter the specimen identifier as the Sample No. for the slot in which the corresponding cartridge will be loaded, and then touch the **"Optional"** button.

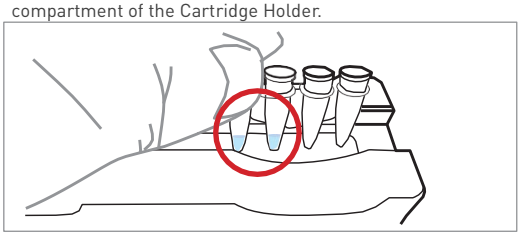


Note: Select only the slot(s) containing a prepared cartridge.

5) Select the **"RP033"** button and touch the **"Yes"** button.

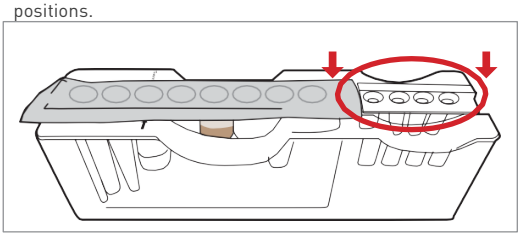
9-2 Cartridge & PCR reagent setup

1) Remove the cap from the Ebola & Marburg Pre-filled PCR Tube and place it in the Cartridge Holder in the indicated orientation. Position Well A in the first compartment and Well B in the second compartment of the Cartridge Holder.

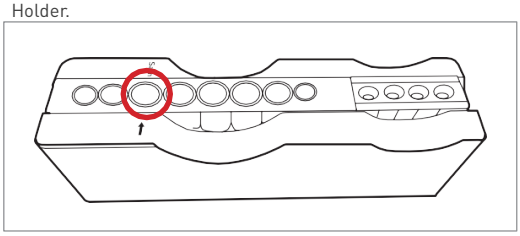


Note: Verify the orientation of the pre-filled PCR tube before connecting it to the extraction cartridge. Well A corresponds to the Ebola virus assay, and Well B corresponds to the Marburg virus assay.

2) Prepare the extraction cartridge and align it correctly with the Cartridge Holder. Securely connect the PCR connectors of the extraction cartridge to the corresponding PCR wells by pushing the connectors fully into place. Do not interchange the Well A and Well B positions.

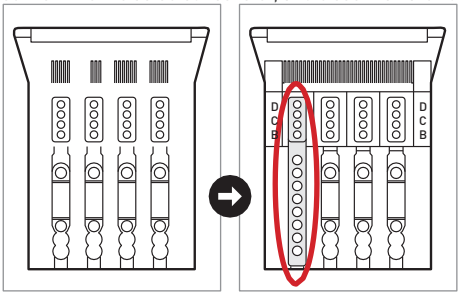


3) Remove the lid of the extraction cartridge and add 200 µl of the sample into the sample compartment indicated on the Cartridge Holder.

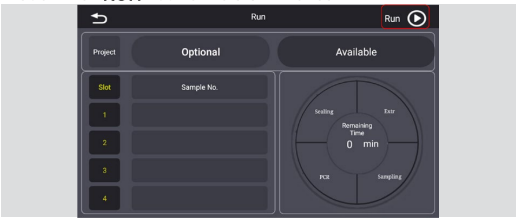


9-3 Load the cartridge and Run

1) Before loading the cartridge, verify that the specimen identifier on the specimen container matches the Sample No. entered for the selected test slot.
2) Open the hatch door, load the cartridge containing the corresponding specimen into the selected test slot, and close the hatch door.



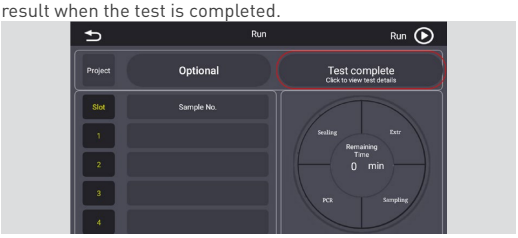
3) Before starting the test, verify that the Sample No. entered for each selected slot corresponds to the specimen and cartridge loaded in that slot.
4) Touch the **"RUN"** button to start the test.



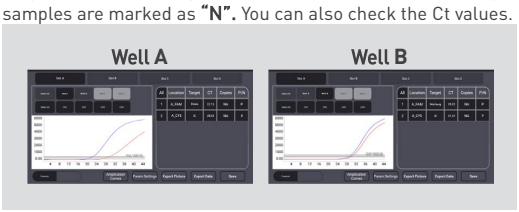
10. Result Check and Interpretation

10-1 Result check

1) Touch **"Test complete"** from the screen in order to check the test result when the test is completed.



2) Amplified positive samples are marked as **"P",** and non-amplified samples are marked as **"N".** You can also check the Ct values.



10-2 Test interpretation

1) The result reported for Well A corresponds to the Ebola virus assay, and the result reported for Well B corresponds to the Marburg virus assay.
2) An "Ebola Positive" result indicates the detection of one or more Ebola virus species within the validated detection scope. The assay does not identify or differentiate the individual Ebola virus species detected.
3) Test interpretation of RADIONE Ebola/Marburg Detection Kit is described as below.

Well A

Case	Target		Result
	Ebola (FAM)	IC (Cy5)	
1	≤40	≤40 or -**	Ebola Positive*
2	>40 or -	≤40	Ebola Negative
3	>40 or -	>40 or -	Invalid***

Well B

Case	Target		Result
	Marburg (FAM)	IC (Cy5)	
1	≤40	≤40 or -**	Marburg Positive
2	>40 or -	≤40	Marburg Negative
3	>40 or -	>40 or -	Invalid***

* An "Ebola Positive" result indicates detection of Ebola virus RNA. The assay does not differentiate among individual Ebola virus species.

** For both Well A and Well B, a positive target result remains valid even if the internal control is not detected.

*** For both Well A and Well B, the result is invalid when neither the target nor the internal control is detected. Repeat the test using new test components. If the repeated result remains invalid, collect a new specimen and repeat the test. Refer to Section 14. Troubleshooting.

11. Quality control

Users may periodically verify the expected amplification and detection performance of the RADIONE Ebola/Marburg Detection Kit (RP033) using the RADIONE Ebola/Marburg Positive Control Kit (RP033-PC), which is supplied separately by the manufacturer.

Control testing should be performed in accordance with the laboratory's quality-control procedures, including when a new reagent lot is introduced, when storage or handling conditions may have been compromised, or when unexpected or inconsistent results are observed.

For detailed control preparation and test procedures, refer to the Instructions for Use supplied with RP033-PC.

The positive control contains synthetic target DNA and verifies reagent amplification and detection performance. It does not evaluate the viral lysis step and is not intended for quantitative calibration or assignment of target concentration in clinical specimens.

Well A	Control	Ebola (FAM)	IC (Cy5)
	NTC	No Ct or Ct>40	No Ct or Ct>40
	PC	18≤Ct≤25	18≤Ct≤25

Well B	Control	Marburg (FAM)	IC (Cy5)
	NTC	No Ct or Ct>40	No Ct or Ct>40
	PC	18≤Ct≤25	18≤Ct≤25

Control results are acceptable when all results meet the criteria specified in the table above.

If the positive control or negative control result is outside the specified acceptance criteria, the run shall be considered invalid. Patient results from the affected run shall not be reported. Investigate the cause of the control failure and repeat the test using new control and test components. If the repeated control result remains unacceptable, discontinue testing and contact KH Medical or its authorized representative.

12. Test Limitations

1) The intended-use specimen type for the RADIONE Ebola/Marburg Detection Kit is limited to human EDTA plasma. Analytical performance, including specimen-related performance characteristics, was established using human EDTA plasma. Other specimen types, including serum, EDTA whole blood, and oropharyngeal swabs, are not included in the intended-use claim.
2) This kit provides qualitative results only and does not provide quantitative values. Ebola virus results are reported collectively as "Ebola Positive" without species-level differentiation.
3) All users, analysts, and any personnel reporting diagnostic results should be trained to perform this procedure by a competent instructor. They should demonstrate their ability to perform the test and interpret the results before performing the assay independently.
4) Negative results do not preclude the presence of Ebola virus or Marburg virus and should not be used as the sole basis for treatment or other patient management decisions. Results should be interpreted together with clinical, epidemiological, and other laboratory findings [1-3].
5) Detection of viral RNA may not indicate the presence of infectious viruses or that the detected virus is the causative agent of the clinical symptoms [1, 2].
6) False positive results may occur due to cross-contamination between patient specimens, specimen mix-up, and/or nucleic acid contamination during product handling.
7) False-negative results may arise from:
   - Improper specimen collection, transportation, or storage.
   - Viral RNA concentrations are near or below the limit of detection of the test.
   - Degradation of viral RNA during specimen transportation and/or storage.
   - Genetic variation or mutations in the target regions that may affect primer or probe binding.
   - Failure to follow the Instructions for Use.
8) The assay detects Ebola virus species within the validated detection scope and reports all detected Ebola virus species collectively as "Ebola Positive." The assay does not differentiate among Zaire, Sudan, Bundibugyo, Taï Forest, and Reston viruses. Therefore, an "Ebola Positive" result must not be interpreted as identification of a specific Ebola virus species. Additional species-specific testing may be required when identification of the individual species is clinically or epidemiologically necessary.

13. Performance characteristics

Analytical sensitivity (Limit of Detection)

In order to determine the limit of detection (LoD) of the RADIONE Ebola/ Marburg Detection Kit, a tentative LoD was established using serially diluted synthetic DNA. After the tentative LoD was established, the claimed LoD was verified using 20 replicates at concentrations around the tentative LoD.

- **Claimed LoD**

Target	Copies/mL
Ebola	800
Marburg	800

- **Cut off Value**

A result with a Ct value of ≤40 is considered positive.

Cross Reactivity

Potential cross-reactivity was evaluated by *in silico* sequence homology analysis using the NCBI BLASTn program. Each assay primer and probe sequence was individually compared with nucleotide sequences of the microorganisms included in the analytical specificity assessment.

Where sequence homology of 80% or greater was identified for an individual primer or probe, the complete forward primer, reverse primer, and probe set was further evaluated to determine whether the combined oligonucleotide set could reasonably support amplification and target-specific fluorescence detection.

Although isolated sequence homology of 80% or greater was identified for individual oligonucleotides with some non-target microorganisms, no complete primer-probe combination capable of supporting cross-reactive amplification and detection was identified. Therefore, the likelihood of cross-reactivity with the evaluated non-target microorganisms was considered low.

Precision

The precision of the RADIONE Ebola/Marburg Detection Kit was evaluated using negative, low-positive, and moderately positive EDTA plasma specimens.

Repeatability was evaluated using 20 replicates per panel member under the same testing conditions, including the same day, operator, instrument, and reagent lot.

Reproducibility was evaluated across three testing days, six runs, two operators, two RADIONE 4ch instruments, and three reagent lots.

All positive specimens were detected, all negative specimens showed no target amplification, and valid internal control results were obtained throughout the study. The Ct value coefficients of variation for repeatability and reproducibility were within the predefined acceptance criterion of 5%. The assay therefore demonstrated acceptable repeatability and reproducibility under the evaluated testing conditions.

Clinical Performance

The clinical performance of the RADIONE Ebola/Marburg Detection Kit was evaluated using archived clinical specimens collected from individuals suspected of Ebola virus disease during the 2026 outbreak response in the Democratic Republic of the Congo. The results obtained with the RADIONE Ebola/Marburg Detection Kit were compared with those obtained using the RealStar® Ebolavirus RT-PCR Kit as the comparator method.

A total of 81 evaluable clinical specimens were included in the final performance analysis, comprising 73 EDTA whole-blood specimens and 8 oropharyngeal swab specimens. Of these, 38 specimens were positive and 43 specimens were negative by the comparator method.

The EDTA whole-blood and oropharyngeal swab specimens were archived clinical specimens available from the clinical study site during the outbreak response and were used solely for the clinical performance evaluation. The intended-use specimen claim for the RADIONE Ebola/Marburg Detection Kit remains limited to human EDTA plasma. Therefore, the clinical study results do not support an intended-use claim for EDTA whole blood, oropharyngeal swab, serum, or any other specimen type.

The clinical performance results are summarized below.

Clinical Performance Measure	Result
Comparator-positive specimens	38
Comparator-negative specimens	43
Positive Percent Agreement	100.0% (38/38)
Negative Percent Agreement	100.0% (43/43)
Overall Percent Agreement	100.0% (81/81)
False-positive results	0
False-negative results	0

No false-positive or false-negative results were observed among the 81 evaluable specimens. The comparator method did not differentiate individual Orthoebolavirus species. Therefore, separate clinical performance estimates for individual Ebola virus species could not be established.

Clinical performance for Marburg virus was not evaluated because confirmed Marburg virus-positive clinical specimens were not available. Therefore, the clinical performance results presented above apply only to the Ebola virus target.

14. Troubleshooting

1) No target signal or invalid result

This kit uses an Internal Control (IC) to verify specimen adequacy and the validity of the nucleic acid extraction and amplification processes. If no target signal is detected but the IC is detected, the result may be interpreted as negative in accordance with Section 10.

If neither the target nor the IC is detected, the result is invalid. Possible causes include insufficient specimen volume, improper specimen collection, transportation or storage, PCR inhibition, incorrect cartridge or PCR tube setup, reagent deterioration, or instrument malfunction.

Repeat the test using the same specimen and new test materials. If the repeated result remains invalid, collect a new specimen and perform a new test. If invalid results continue after specimen recollection and repeat testing, discontinue testing and contact KH Medical or its authorized representative for technical support.

2) Control failure

If a positive or negative control result does not meet the acceptance criteria specified in Section 11, the run is invalid. Do not report patient results from the affected run. Check the control preparation, reagent condition, storage conditions, test setup, and instrument status, and repeat the control and affected specimen tests using new control and test materials.

If the control failure persists, discontinue testing and contact KH Medical or its authorized representative for technical support.

3) Suspected reagent instability or deterioration

Do not use reagents or consumables that are expired, improperly stored, damaged, leaking, discolored, contaminated, or otherwise suspected of deterioration. Replace the affected materials with new, intact materials and repeat the test.

If deterioration is suspected for multiple components or the cause cannot be resolved, discontinue use of the affected kit lot and contact KH Medical or its authorized representative.

4) Instrument malfunction

If an instrument error, abnormal operation, or repeated system warning occurs, stop testing and follow the troubleshooting instructions provided in the RADIONE 4ch Instrument user manual. Do not report results from an affected run unless the run has been completed and confirmed as valid.

If the malfunction cannot be resolved, discontinue use of the instrument and contact KH Medical or its authorized representative.

5) Unexpected or inconsistent result

If a result is unexpected or inconsistent with the clinical or epidemiological information, review the specimen identification, specimen condition, collection and storage history, reagent condition, test preparation, control results, and instrument status.

Repeat the test using the same specimen and new test materials when sufficient specimen remains and no specimen-quality issue is identified. Collect a new specimen when the original specimen does not meet the acceptance criteria, when specimen integrity is uncertain, or when repeated testing produces an invalid or inconsistent result.

Additional confirmatory or species-specific testing may be considered where clinically or epidemiologically appropriate.

Technical support

For unresolved control failures, repeated invalid results, suspected product deterioration, persistent instrument malfunction, or other technical issues, discontinue testing and contact KH Medical at **adamhong@khmedical.co.kr**

15. Bibliography

[1] World Health Organization (WHO)

Ebola disease.
https://www.who.int/news-room/fact-sheets/detail/ebola-disease

[2] World Health Organization (WHO)

Marburg virus disease.
https://www.who.int/news-room/fact-sheets/detail/Marburg-virus-disease

[3] Centers for Disease Control and Prevention (CDC)

Clinical Testing Guidance for Patients with Suspected Viral Hemorrhagic Fevers.
https://www.cdc.gov/locs/2023/12-11-2023-lab-advisoryclinical_testing_guidance_patients_suspected_viral_hemorrhagic_fevers.html

16. Description of Symbol Used

Symbol	Description	Symbol	Description
REF	Catalogue number		Consult instruction for use
LOT	Lot number	-25°C 15°C	Storage at -25°C to -15°C
	Use by date		Manufacturer
Σ	Contains sufficient for tests	IVD	In vitro diagnostic Medical Device
	Date of manufacture		

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